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PRÉSENCE D'ENDOTOXINE DAN LES PRÉPARATIONS DE RIBOSOMES ET ANTIGÉNICITÉ DES ACIDES NUCLÉIQUES

PRESENCE OF ENDOTOXIN IN RIBOSOMAL PREPARATIONS AND THE ANTIGENICITY OF NUCLEIC ACIDS

by J.-P. Dandeu, G. Quash, and E. Barbu

In order to immunochemically characterize ribosomes, animals were inoculated with ribosomal preparations from various bacteria. These preparations were obtained by the method of Tissières and Watson[1], by crushing the bacteria in 0.01 M magnesium acetate in 0.005 M Tris buffer (pH 7.5). The ribosomes were partially purified by sedimenting them four times by centrifugation at 100,000 x g.

In the course of research on the different antigenic determinants of ribosomes, it was confirmed that, in addition to ribosomes, the preparations contained some particles which were not sedimented at 100,000 x g but were devoid of RNA.

These particles have been identified as being endotoxin (O antigen[2]) of gram negative bacteria by the following procedures: phosphorous and nitrogen content, anthrone positive carbohydrate content, reaction with O-antisera, ultraviolet absorption spectra (maximum at 280 or 260 mμ), and the reaction of animals injected with the particles.

The endotoxin has been isolated from ribosomal preparations by precipitation of the ribosomes with streptomycin. The endotoxin in the supernatant is removed by centrifugation at 70,000 x g. It is then dissolved in distilled water and purified by centrifugation on a sucrose gradient. After purification, it gives a negative reaction with the Folin-Ciocalteu reagent of Lowry et al.[3] used for protein determinations.

The presence of endotoxin in the ribosomal preparations results consequently in the production of anti-endotoxin antibodies in the anti-ribosomal sera and leads to a number of problems:

(1) Mishaps of Immunization--In the course of animal immunization with ribosomal preparations, the death of the animal may be observed without pathological disorders. It is possible that this is due in part to the presence of endotoxin in the preparations.

(2) Antigenicity of Nucleic Acids-- Anti-RNA antibodies can be demonstrated in antisera towards bacterial ribosomes[4]. In order to determine exactly the influence of ribosomal origin on the formation of these antibodies, rabbits have been immunized with ribosomes obtained from different bacterial species, with ribosomal extracts from rabbit or rat liver, or with ribosomes from ascites cells.

Antibodies precipitating with RNA have been obtained in the sera of rabbits inoculated with ribosomes from Escherichia coli, Salmonella typhimurium, Proteus vulgaris, and Hemophilus influenzae 'B'. These antisera contained from 300 to 700 µg per ml of antibody precipitating with RNA. In the case of antisera against the ribosomes of Streptococcus fecalis, Staphylococcus aureus, Welchia (Clostridium)perfringens, Alcaligenes fecalis, and ribosomes of animal origin, there were no antibodies which precipitated with ribosomal RNA or with polyadenylic acid (poly A). The same was observed with sheep antisera against C. perfringens and A. fecalis.

It was noticed, however, that all of the latter sera, that do not react with RNA, gave feeble reactions (100 to 200 µg antibody per ml serum) with heterologous ribosomes although the ribosomes were obtained from bacterial species which were quite different from the cells which were used to obtain ribosomes for immunization.

A good anti-RNA titer was obtained with a serum of a goat immunized with E. coli ribosomes but the highest titer was obtained with a horse immunized with P. vulgaris ribosomes: 1 mg antibody per ml of serum.

Ribosomal preparations which yielded antisera richest in anti-RNA antibodies were obtained from cells having endotoxin. The question arises as to whether it is the structure of the ribosomes or the presence of endotoxin that is responsible for these differences. With regard to the latter, there is the question as to its role:

(A) Endotoxin may play the unique and well known role of non-specifically stimulating the production of antibodies directed against RNA specifically.

(B) The endotoxin may bear an antigenic stie which induces the synthesis of antibodies which, in addition to reacting with this site, cross react with RNA. The latter possibility is not probable since endotoxins isolated and purified from ribosomal preparations of E. coli, S. typhimurium, and P. vulgaris do not react with antibodies isolated from horse serum against P. vulgaris ribosomes using poly A. In contrast, as was previously demonstrated[5], these antibodies are completely precipitated by RNA, polyribonucleotides, and ribosomes.

The serum of a rabbit which received E. coli ribosomes free of endotoxin as a result of streptomycin precipitation, does not give a precipitin reaction with RNA. In contrast, sera from rabbits immunized with a conventional ribosomal preparation for the same length of time does give a reaction. This indicates that the role of endotoxin is that of a nonspecific stimulant in the production of anti-RNA antibodies.

This being the case, one is forced to ask the question as to the possible role of endotoxin in the stimulation of anti-DNA antibodies in the case of diseases such as dessiminated lupus erythematosus.

(3) Anti-RNA Antibodies and Cross Reactions-- In a previous note[6] it was demonstrated that under certain conditions, anti-RNA antibodies will precipitate with other negatively charged polyelectrolytes such as DNA, heparin, and dextran sulfate. If one takes into account the fact that the antisera was obtained by immunization with ribosomes and was isolated by precipitation with poly A (fraction a) and also with poly I (fraction b), then it is possible to assume that these latter precipitin reactions are also cross reactions and that RNA reacts for the same reason with the two antibody fractions.

In order to explain the existence of all these cross reactions, there are two hypotheses: (a) the polyribose phosphate moiety is responsible for the cross reactions, (or) (b) the anti-RNA antibody comprises a separate class of γ-globulins which is more basic than the other γ-globulins found in serum[6].

The existence of cross-precipitation reactions with anti-RNA antibodies poses the next problem: how to the other substrates of these reactions manifest themselves? This problem is even more critical as can be seen when one considers the presence in the sera of nonimmunized rabbits of antibodies which precipitate with poly I. Do antibodies against endotoxin and other polysaccharides give general gross reactions with RNA or polynuclectides?

(4) Presence in Human Sera and in Nonimmunized Animals of γ-Globulin reacting with RNA-- Evidence has been presented for the existence of γ-globulins isolated from sera by precipitation with poly I. The γ-globulins isolated in this manner interact strongly with ribosomal RNA but do not give a precipitin reaction. On the other hand, they give a weak reaction of precipitation with TMV RNA.

At present, one can distinquish the following classes:

(a) Antibodies reacting strongly with RNA.

(b) γ-globulins showing interactions with RNA.

(c) Antibodies directed against other antigenic sites but which also give a precipitin reaction with RNA.

Because of the difficulties that are encountered from the point of view of characterizing the specificity of these γ-globulins, another area of investigation comes to light, namely, the role of these γ-globulins in the nonspecific defenses of the host. In fact, it possible that these γ-globulins are able to act as opsonins at the onset of microbial or viral infections because of their strong interactions with different negatively charged polyelectrolytes.

Numerous authors[8] have demonstrated the opsonizing ability of these serum globulins without any immunization. Their specificity, however, is not precise.

In any case, if the interaction of these γ-globulins which react with poly I proves favorable to a host, we know how to stimulate their formation and also how to separated and concentrate them.

Their ability to fix complement[9] is also important. Similarly, one can cite the case of properdin which is characterized by its ability to precipitate with zymosan polysaccharide[10].

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